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RESEARCH ARTICLE

ICH Q2(R1)-GUIDED VALIDATION OF ANORMAL PHASE HPLC/UV METHOD FOR THIRAM IN TECHNICAL WP FORMULATIONS COMPLYING WITH SANCO QC STANDARDS

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Abstract

To develop and validate a robust normal phase HPLC UV method for quantifyi ng thiram in its formulations, ensuring it meets validation criteria defined in ICH Q2(R1) and residue limits specified by SANCO (SANCO/12571/2013 rev. 3).Silica-based normal-phase column with an optimized mixture of non polar protic solvents (e.g., hexane/isopropanol) delivering strong retention and sharp UV-detectable peaks. UV detection set at thiram's λ_max (typically ~230–254 nm), optimized during method development.Thiram extracted from the 80% WP matrix via solvent extraction and centrifugation, followed by clean-up to minimize matrix interferences.Verified by injecting blank (solvent), placebo (matrix without API), spiked sample, and reference standard—confirming no co-eluting peaks at thiram's retention time; peak purity confirmed via UV spectral matching. Calibration curve across 80–120% of nominal concentration with ≥ 5 concentration levels; correlation coefficient (r^2) ≥ 0.998.Performed at three spike levels (80%, 100%, 120%); recoveries between 98 102%.Precision Repeatability(intra day)RSD ≤ 2% and Intermediate(inter day)RSD ≤ 3%, confi rming reproducibility.LOQ & LODdetermined by using signal-to-noise (S/N) and calibration slope per ICH guidelines; LOQ meets or surpasses the SANCO-required 0.01 mg/kg for plant matrices. Method tolerance tested against minor deliberate changes (e.g., ±5 °C column temp or ±0.1 mL/min flow); RSD remained ≤ 3%.Processed sample and standard solution stability confirmed for ≥48 hours (4 °C) and two weeks (refrigerated), respectively.Verified by parame ters including retention time, theoretical plates, tailing factor, and reproducibilit y via repeat standard injections. The method's LOQ (≤ 0.01 mg/kg) adheres to high residue levels for dry crops and WP formulations . Supports robust quantif ication for regulatory enforcement in food/feed and environmental matrices. The developed normal-phase HPLC UV method isvalidated as per ICH Q2(R1) and SANCO guidelines demonstrating specificity, accuracy, precision, sensitivi ty, and robustness. It is suitable for routine regulatory analysis of thiram 80% WP and its residues across diverse matrices.

Introduction:-

Thiram is a multi-purpose fungicide, seed treatment, and industrial additive widely used in agriculture and rubber processing. While effective at preventing fungal disease and deterring animals, it can be toxic, particularly to the nervous, reproductive, and sometimes cardiac systems in animals—and is harmful to aquatic life. Proper handling,

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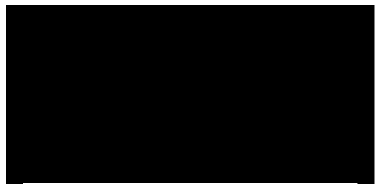
protective gear, and awareness of safety guidelines are essential.Historically used in treatments for human scabies, as a mild bactericide or sunscreen ingredient, and in textile and paper manufacturing. Used to coat seeds and prevent fungal diseases (e.g., damping off, smut, scab) in crops and turf. Thiram is also used as a sulfur source and secondary accelerator the sulfur vulcanization (accelerate sulfur curing of rubber) of rubbers. Coated on fruits, ornamentals, and seeds to deter rabbits, rodents, deer, birds, etc.High doses in animals caused infertility, embryo toxicity, birth defects such as cleft palate. Some chromosomal damage and mutagenic effects in rodent and cell studies, though evidence is mixed. In poultry and fish embryos, thiram induced oxidative stress, apoptosis, and developmental abnormalities. It also have a characterof an antibacterial and antiseptic drug. It contains a dimethyldithiocarbamate.

Thiram (IUPAC: dimethylcarbamothioicdithioperoxyanhydride; CAS 137-26-8) is the simplest thiuram disulfide, chemically an oxidized dimer of dimethyldithiocarbamate. It's a white-to-grey/cream powder (melting point ~155 °C), poorly soluble in water (~30 mg/L), and has a slight characteristic odor.



Chemical structure of thiram

Oral LD_{50} ranges from ~210 to 1,350 mg/kg across species; inhalation LC_{50} (4 h, rats) ~500 mg/m³

The analytical method of the determination of active ingredient content Thiram of Thiram 80% WP was validated by analyzing the test substance and reference standard. The validation covered the aspects namely (i) Specificity, (ii) Linearity, (iii) Limit of detection (LOD), (iv) Limit of quantitation (LOQ), (v) Precision (% RSD) and (vi) Accuracy (% Recovery).

Study Objective& Guideline

This study was performed to validate the analytical method for active ingredient analysis of Thiram WP formulation. This study was conducted in compliance with OECD principles of GLP (1998).Validation of the analytical method for active ingredient analysis of Thiram technical was determined as per method described in International Conference on Harmonisation (ICH-Q2(R1)).

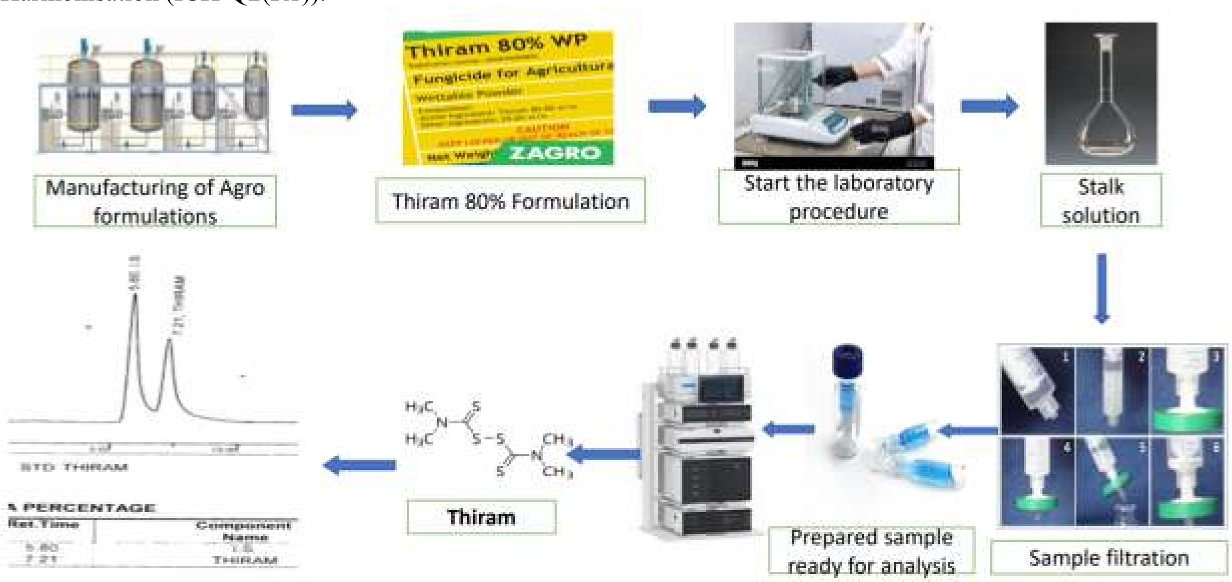


Fig: Thiram Method development Procedure

Experimental

Chemical and reagents

HPLC grade reagents and chemicals (Hexane, Isopropanol, DCM) were used throughout the experiment. Deionized water was used for the preparation of all the solutions. The standards and formulations of thiram were obtained from the Department of Chemistry, Institute For Industrial Research And Toxicology, Ghaziabad, India.

Instrumentation

Chromatographic analysis was done on a 1220 HPLC with UV-VIS detector (Agilent 1220 Infinity single Pump stands out as the preferred pump for achieving consistent isocratic and optimal performancein scenarios requiring high throughput and rapid separations. The 1220infinity HPLC system having a manual injection features couples with advance EZ Chrome software for data generation and calculation.

AHypersil Silica (250 mm X 4.6 mm, 5µ particle size)column wasused for the stationary phase.Integration of chromatographic analysis was achieved with aAgilent UV detector (Agilent Technology USA), equipped with a communication bus module, and data were evaluated on Chromatography software EZ Chrome for Windows workstation latest version software, Agilent Technology USA).

HPLC Condition and Determination of λmax

Solvent for mobile phase was initially tested by analyte solubility in methanol, water and acetonitrile, DCM, Hexane and isopropanol. Both solvents provided acceptable solubility accept methanol water and ACN; therefore, different ratios of hexane and isopropanol were checked to optimize the mobile phase for a good separation of analytes with the highest resolution. To obtain the shortest retention time without losing the optimized chromatographic response of the analyte, the mobile phase was tested at different flow rates. The separation was accomplished with a Hypersil Silica (250 mm X 4.6 mm, 5µ particle size)column at ambient temperature. Isocratic mode of mobile phase fixed for Chromatographic separation analysis.

For the determination of λmax using Micro Processor UV - Visible Spectrophotometer Double Beam Model SS-2700. Solution was prepared by dissolving accurately weighed quantity of 10.0 mg of standard and diluting to 100 ml in a volumetric flask with mobile phase. The UV absorption was taken in the range of 200 nm to 400 nm using Mobile phase as blank. The UV exhibits an absorbance peak at the wavelength of 233 nm and it corresponds to the UV spectra obtained with sample solution prepared in the similar manner. The instrument operation condition was: Bandwidth: 0.5 nm, Mode: Scan, Scan speed Slow.

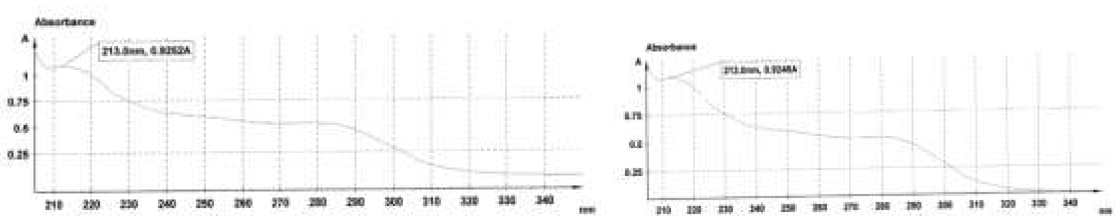


Fig: λmax Determination of thiram standard and formulation

Validation of Analytical Method

The analytical method for the determination of active ingredient content of ThiramWP was validated by analyzing the test substance and the reference standard using normal phase HPLC method with slight modification [CIPAC 24/TC/M3/-(CIPAC Hand book D, p.169)]. The validation covered the aspects of (i) specificity (ii) Linearity (iii) Limit of detection (LOD) and Limit of quantitation (LOQ) (iv) Precision (% RSD) and (v) Accuracy (Recovery%).

For the demonstration of any analytical procedure which is using in the analytical purpose have important to evaluation of validation parameters viz: analytical curve and linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy (%RSD), recovery, precision (repeat ability and intermediate precision), and specificity. This method validation procedure proof and confirm that this method is very suitable for its intended use.

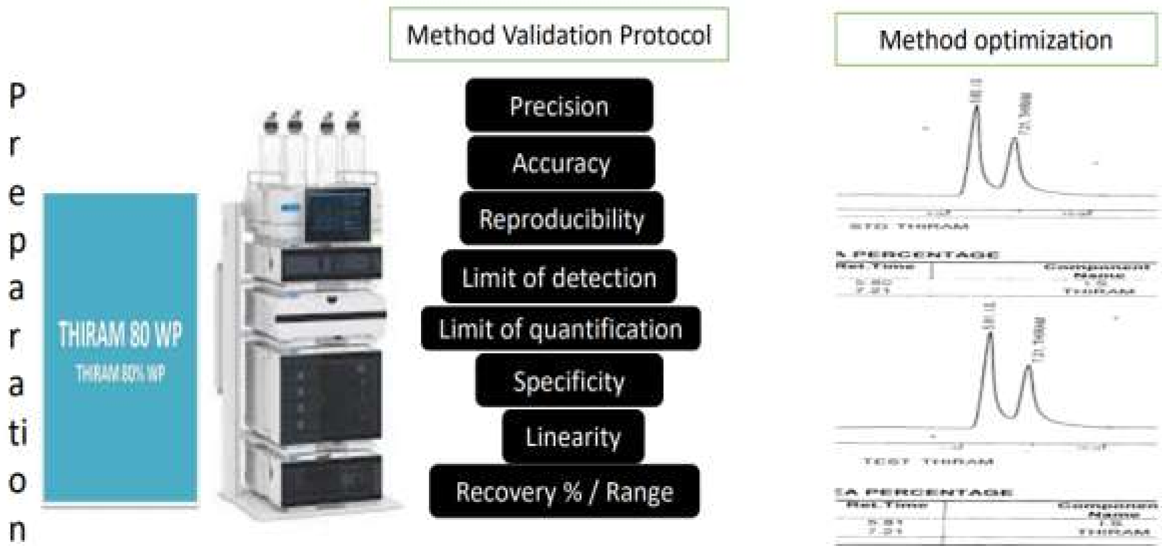


Fig: Method Validation Protocol of Thiram Formulation

Mobile-Phase Composition

Different mixtures of Hexane and isopropanol (HPLC grade) in different compositions was checked for fine separation and bright resolution. The mobile-phase composition that had good separation and the lowest retention time was hexane: isopropanol (95:5, v/v).

Flow Rate

Mobile-phase flow rates were studied in the range of 0.8 to 1.5 mL/min and after suitable adjustment of pH and getting a good result in separation, fix the flow rate 1.2 mL/Min, fine resolution.

Specificity

Specificity of HPLC method for active ingredient analysis was studied by injecting Thiram Reference Standard solution, Formulation solution, Dichlormethane(Solvent used for solution preparation),and mobile phase for any interference between components, with each other or with any of their components.

Preparation of StandardStock Solutions

Component	Weight taken (mg)	Purity	Volume of Internal Standard	Final Volume	Concentration Mg/L	Standard Stock Solution
Thiram reference std	6.4	99.5	-	25	256	A
Internal Standard	7.5	99.0	-	25	300	B
Thiram Std. mixture	0.128 (0.5 ml A)	-	0.5 ml B	10	10	C

Stock solution were prepared using DCM and further dilution were also made using DCM.

Preparation of Formulation solution

<table>
<tr><th rowspan="2">S.No.</th><th rowspan="2">Weight (mg) of Formulation</th><th rowspan="2">Final Volume (ml)</th><th rowspan="2">Volume made using</th><th colspan="4">Dilution of solution</th></tr>
<tr><th>Solution Taken (ml)</th><th>I.S. added (ml)</th><th>Final Volume (ml)</th><th>Volume made using</th></tr>
<tr><td>1</td><td>8.29</td><td rowspan="5">25</td><td rowspan="5">DCM</td><td rowspan="5">0.5</td><td rowspan="5">0.5</td><td rowspan="5">10</td><td rowspan="5">DCM</td></tr>
<tr><td>2</td><td>7.92</td></tr>
<tr><td>3</td><td>7.81</td></tr>
<tr><td>4</td><td>7.78</td></tr>
<tr><td>5</td><td>7.92</td></tr>
</table>

Linearity

Preparation of Thiram Reference Standard Solutions for linearity

Reference Standard Stock Solution	Solution Taken (mL)	I.S.Stock Solution	Solution Taken (mL)	Final Volume(mL)	Volume made using	Obtained Conc. (mg/L)	Identification
A	0.5	B	0.5	10	DCM	12.8	L1
	1.0		0.5			25.6	L2
	2.0		0.5			51.2	L3
	3.0		0.5			76.8	L4
	4.0		0.5			102.4	L5

The reference standard solution L1, L2, L3, L4 and L5, were injected onto HPLC in duplicate using the parameters in accordance with validation protocol and the mean peak area was plotted against concentration (mg/L). The correlation coefficient R and intercept with y-axis were calculated and the regression equation y= bX + a was established.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Preparation of Thiram Reference Standard solutions for LOD and LOQ

Reference Standard Solution	Solution Taken (mL)	I.S. Stock Solution	Solutin Taken (mL)	Final Volume (mL)	Volume made using	Obtained Conc. (mg/L)	Identification of Reference Standard Solution
A	0.25	B	0.5	10	DCM	6.4	DQ1
	0.5		0.5	10		12.8	DQ2
	1.0		0.5	10		25.6	DQ3

The reference standard solutions (DQ1, DQ2 and DQ3) were injected onto HPLC in duplicate using the parameters in accordance with section 2. The minimum concentration which could be detected by HPLC with signal (Mean Response Factor : Area of Thiram/Area of I.S.) to noise ratio (S/N) of 3:1 was considered as LOD. The minimum concentration which could be quantified with signal to noise ratio (S/N) between 5:1 and 10:1 was considered as LOQ. The average signal: noise ratio was calculated by taking the noise obtained in blank (mobile phase) injections.

Precision (% RSD)

Preparation of Thiram Reference Standard Solution

The reference standard solution (L1), concentration 12.8 mg/L, prepared for linearity was used for precision.

Preparation of Formulation solutions

S.No.	Weight (mg) of Formulation	Final Volume (mL)	Volume made using	Dilution of solution			
				Solution Taken (ml)	Volume of I.S. (B) added (ml)	Final Volume (ml)	Volume made using
1	8.29	10	DCM	0.5	0.5	10	DCM
2	7.92						
3	7.81						
4	7.78						
5	7.92						

The above prepared reference standard solution (L1) and Formulation solutions were injected in triplicate into HPLC using parameters in accordance with validation protocol.

Calculation of Precision (% RSD)
The precision (% RSD) was calculated using following formula:Standard Deviation
Precision (% RSD) = ---------------------- X 100 Mean

Accuracy (% Recovery)
Preparation of Thiram Reference Standard Solution
The reference standard solution (L1), concentration 12.8 mg/L, prepared for linearity was used for accuracy.

Preparation of FormulationSolutions

Level	Replication	Weight (mg) of Formulation	Final Volume of Stock Solution (mL)	Reference Standard Solution used/Weight (mg) of Standard per mL	Volume (mL) added / weight (mg) of Standard	[Quantity Fortified] (%) B	Dilution of Solution	
							Solution Taken (mL)	Final Volume (mL)
I	R1	8.09	25	L1 [0.256]	0.3 mL [0.0768]	0.945	0.5	10
	R2	7.96	25			0.960	0.5	10
	R3	7.93	25			0.964	0.5	10
	R4	8.01	25			0.954	0.5	10
	R5	8.10	25			0.943	0.5	10

Typical Calculation

Calculation of Quantity Fortified (%)

$$= \frac{\text{Weight (mg) of reference standard}}{\text{Weight (mg) of Formulation}} \times \text{Purity of Reference Standard} \qquad = \frac{0.0768}{8.09} \times 99.5 = 0.945$$

Preparation of Formulation Solutions (Continued)

Level	Replication	Weight (mg) of Formulation	Final Volume of Stock Solution (mL)	Reference Standard Solution used/Weight (mg) of Standard per mL	Volume (mL) added / weight (mg) of Standard	[Quantity Fortified] (%) B	Dilution of Solution	
							Solution Taken (mL)	Final Volume (mL)
II	R1	8.15	25	L1 [0.256]	0.6 mL [0.1536]	1.875	0.5	10
	R2	8.17	25			1.871	0.5	10
	R3	8.17	25			1.871	0.5	10
	R4	8.22	25			1.859	0.5	10
	R5	8.17	25			1.871	0.5	10

Typical Calculation

Calculation of Quantity Fortified (%)

$$= \frac{\text{Weight (mg) of reference standard}}{\text{Weight (mg) of Formulation}} \times \text{Purity of Reference Standard} \qquad = \frac{0.1536}{8.15} \times 99.5 = 1.875$$

Results & Discussion:-

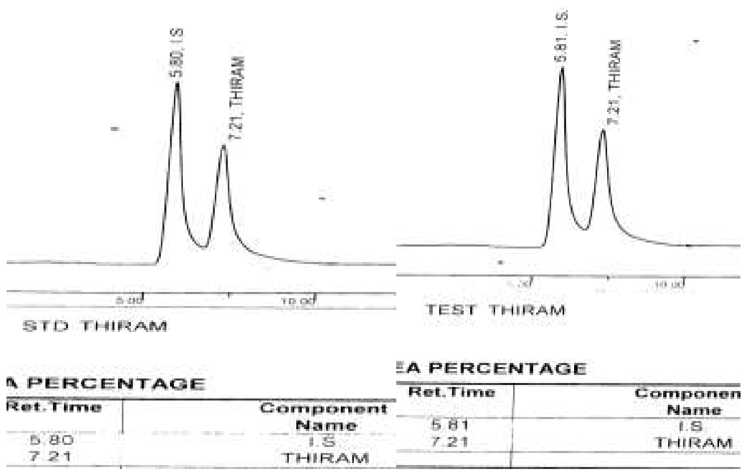
Validation of HPLC Analytical Method
The analytical method for determination of active ingredient content of Thiram 80% WP was validated. The validation covered the aspects namely; (i) Specificity (ii) Linearity (iii) Limit of detection (LOD),Limit of quantitation (LOQ), (iv) Precision (% RSD) and (v) accuracy {% recovery}.

Specificity

Specificity of the assay was established by finding chromatograms for blank and observing the lack of nosy peaks at the retention time for the compounds. Specificity was performed to compare the standard and formulations of thiram. It was calculated by. inject a specificity standard solution to evaluate and ensure the separation actives. The parameters measured will be retention time (RT) that will be calculated directly by software.

Table 2A: Specificity report format

Average Response (RT)				Average Response (RT)			
Thiram Standard RT	Internal Standard RT	% RSD triplicate injections		ThiramFormulation RT	Internal Standard RT	% RSD triplicate injections	
7.21	5.80	0.42%	0.39%	7.21	5.81	0.57%	0.50%
7.19	5.81			7.18	5.76		
7.20	5.83			7.28	5.79		
7.24	5.81			7.22	5.83		
7.26	5.80			7.19	5.84		
7.18	5.86			7.27	5.82		



A. ***Fig:*** **Chromatograms showing RT for thiram standard and formulation with Internal Standard**

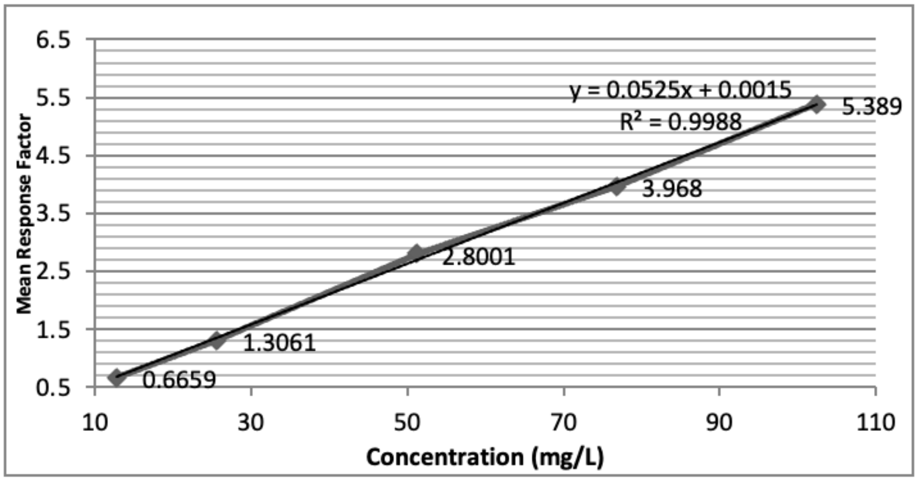
Analytical curve and linearity

The linearity of the method was established by injecting five different concentrations viz. 12.8, 25.6, 51.2, 76.8 and 102.4 mg/L of Thiram reference standard solutions onto HPLC in duplicate and plotting the mean peak area against concentration (mg/L). The correlation coefficient R was 0.998.

Table1: Linearity table of Thiram reference standard.

Concentration (mg/L)	Replication	Peak Area of Thiram	Peak Area of Internal Standard	Response Factor	Mean Response Factor	% Variation
12.8	I	939.3034	1415.1742	0.6637	0.6659	0.64
	II	949.6047	1421.7938	0.6680		
25.6	I	2056.2311	1568.3863	1.3110	1.3061	0.76
	II	2041.1522	1568.7015	1.3012		
51.2	I	3881.0163	1390.6257	2.7908	2.8001	0.65
	II	4019.2331	1430.6831	2.8093		
76.8	I	6189.9585	1562.4967	3.9616	3.9680	0.32
	II	6213.5618	1563.4239	3.9743		
102.4	I	8387.9097	1557.1489	5.3867	5.3890	0.09

	II	8269.6953	1533.9092	5.3913		
% Variation = Maximum Response Factor-Minimum Response Factor / Maximum Response Factor × 100					= (0.6680-0.6637) / 0.6680 ×100	=0.64%



Linearity Curve ofThiram Reference Standard

Intercept with y-axis (a) = 0.001
Slope of the line (b) = 0.052
Correlation co-efficient or 'r' value = 0.998
Equation :Y = bX + a
Y = 0.052X + 0.001

Limit of Detection (LOD)

The limit of detection (LOD) was determined by injecting the Thiram reference standard solutions of various concentrations (6.4, 12.8 and 25.6 mg/L) [in duplicate]. The minimum concentration which could be detected with Signal(Mean Response Factor : Area of Thiram/ Area of I.S.)to noise ratio (S/N) 3:1 was considered as LOD. The minimum detectable concentration (LOD) determined with signal to ratio (S/N) of 4.08 was 6.4 mg/L.

Limit of Quantitation (LOQ)

The limit of quantitation (LOQ) was determined by injecting the Thiram reference standard solutions of various concentrations (6.4, 12.8 and 25.6) [in duplicate]. The minimum concentration which could be quantified with Signal (Mean Response Factor – Area of Thiram/ Area of I.S.) to noise ratio (S/N) between 5:1 and 10:1 was considered as LOQ. The minimum quantifiable concentration (LOQ) determined with Signal to noise ratio (S/N) of 8.9 was 12.8 mg/L.

TABLE 2: Limit of Detection and Limit of Quantitation of Thiram

Concentration (mg/L)	Peak Area of Thiram	Peak Area Of Internal Standard	Response Factor	Mean Response factor (MRF)	Signal to Noise Ratio (MRF to Blank Ratio)	Remark
6.4	411.7632	1359.7269	0.3028	0.3055	4.08	LOD
	441.7508	1433.7914	0.3055			
12.8	939.3034	1415.1742	0.6637	0.6659	8.90	LOQ
	949.6047	1421.7938	0.6680			
Replication	Noise Area of Blank					Average
	1	2		3		
I	0.0720	0.0999		0.0526		0.0748
Typical Calculation						
				Limit of Detection		Limit of Quantitation

Signal to Noise Ratio= (MRF to Blank Ratio) $= \frac{\text{Response Factor}}{\text{Average Noise Area of Blank}}$		$= \frac{0.3055}{0.0748} = 4.08$	$= \frac{0.6659}{0.0748} = 8.90$
Limit of Detection	6.4 mg/L	Limit of Quantitation	12.8 mg/L

Precision (% RSD)

Precision of the analytical method was determined by analyzing 5 replicate preparations of test substance solutions and assayed for active ingredient content of test substance in each replicate. The mean Thiram a.i. content was 80.5% and the precision (% RSD) was 0.07%.

TABLE 3: Calculation of Precision (%RSD) for A.I. Determination

Replic-ation	Weight (mg) of FormulationW	Peak Area of Formulation	Peak Area of IS in Formulation	Response Factor for Formulation RF	Peak Area of Reference Standard	Peak Area of IS	Response Factor For Standard RF'	Mean Response Factor of Standard $RF`_{ave}$	Thiram A.I. Content (%w/w)	Mean A.I. Content (%w/w)
I	8.29	800.5998	1081.3190	0.7404	726.6345	1025.4021	0.7086	0.7055	80.62	80.52
		821.2335	1111.6686	0.7387					80.43	
II	7.92	873.8503	1238.6723	0.7055	771.0029	1097.8255	0.7023		80.40	80.51
		859.8003	1215.4927	0.7074					80.62	
III	7.81	990.8123	1387.0401	0.7143	843.1926	1178.1549	0.7157	0.7180	81.12	80.49
		1016.7595	1445.6632	0.7033					79.87	
IV	7.78	1017.1397	1444.8190	0.7040					80.25	80.55
		1043.4832	1471.5600	0.7091	952.1818	1321.9519	0.7203		80.84	
V	7.92	868.3066	1209.7220	0.7178					80.38	80.64
		1058.7032	1465.2710	0.7225					80.8791	
Mean										80.54
Standard Deviation (SD)										0.06
Relative Standard Deviation (%RSD)										0.07
Purity of Standard(P)			99.50%	Weight of Std.(W`)		6.400 mg	Dilution Factor			1.00
Typical Calculation										
Thiram A.I. Content (%w/w)						Precision(%RSD)				
$= \frac{RF \times W` \times P}{RF`ave \times W} \times D$ $= \frac{0.7404 \times 6.40 \times 99.50}{0.7055 \times 8.29} \times 1.00 = 80.62\ \%$						$= \frac{StandardDeviation}{MeanContent} \times 100$ $= \frac{0.06}{80.54} \times 100 = 0.07\%$				

Accuracy (% Recovery)

Accuracy of the analytical method was determined by analyzing solutions of test substance fortified for level I (~ 0.95 %) and II (~ 1.89 %) with Thiram reference standard in five replicates. The accuracy (% recovery) was determined by using standard addition method. The mean accuracy (% recovery) was 99.7 for level I and 101.6 % for level II.

Table 4: Calculation of Accuracy (%Recovery) for A.I. Determination
Level 1

Replic-ation	Weight (mg) of FORMULATION W	Peak Area of FORMULATION	Peak Area of IS in FORMULATION	Response Factor for FORMULATION RF	Peak Area of Reference Standard	Peak Area of IS	Response Factor For Standard RF'	Mean Response Factor of Standard $RF`_{ave}$	Thiram A.I. Content (%w/w)	Mean A.I. Content (%w/w) [C]
I	8.09	1384.4094	1902.6585	0.7276	684.1941	972.2216	0.7037	0.7031	81.46	81.49
		1355.4432	1861.5904	0.7281					81.51	
II	7.96	1342.9087	1871.8576	0.7174					81.63	81.50
		1345.1675	1880.7645	0.7152					81.38	
III	7.93	1373.9096	1926.6312	0.7131					81.44	81.49
		1298.4658	1818.9365	0.7139	712.9130	1015.0130	0.7024		81.54	
IV	8.01	1290.2594	1789.7539	0.7209					81.51	81.49
		1339.2437	1858.7906	0.7205					81.47	
V	8.10	1409.3833	1935.1485	0.7283					81.44	81.49
		411.4094	564.1899	0.7292					81.54	
Mean										81.49
Standard Deviation (SD)										0.01
Relative Standard Deviation (%RSD)										0.01
Purity of Standard(P)	99.50%	Weight of Std.(W`)	6.40 mg	Dilution Factor						1.00

Typical Calculation

Thiram A.I. Content (%w/w)	Precision(%RSD)
$= \frac{RF \times W` \times P}{RF`ave \times W} \times D$ $= \frac{0.7276 \times 6.40 \times 99.50}{0.7031 \times 8.09} \times 1.00$ = 81.46 %	$= \frac{Standard Deviation}{Mean Content} \times 100$ $= \frac{0.01}{81.49} \times 100$ = 0.01%

Calculation of Accuracy (%Recovery)

Replication	Actual Thiram Content (%w/w) [A]	Spiked Thiram Content (%w/w) [B]	Total Content After Spiking (%w/W) [A+B]	Actual Recovered Spiked Content (%w/w) [E=C-A]	Accuracy (% Recovery) [E/B× 100]
I	80.54	0.945	81.48	0.945	100.08
II		0.960	81.50	0.962	100.20
III		0.964	81.50	0.950	98.63
IV		0.954	81.49	0.951	99.65
V		0.943	81.48	0.945	100.21
Mean					99.75
Standard Deviation					0.67
Relative Standard Deviation (%RSD)					0.67

TABLE 4 (Contd…..): Calculation of Accuracy (%Recovery) for A.I. Determination
Level 2

Replic-Ation	Weight (Mg) Of Formulation W	Peak Area Of Formulation	Peak Area Of Is In Formulation	Response Factor For Formulation Rf	Peak Area Of Reference Standard	Peak Area of IS	Response Factor For Standard RF'	Mean Response Factor of Standard $RF`_{ave}$	Thiram A.I. Content (%w/w)	Mean A.I. Content (%w/w) [C]
I	8.15	734.7572	988.9288	0.7430	973.4082	1374.8202	0.7080	0.7039	82.48	
		729.5366	982.1440	0.7428					82.45	82.46
II	8.17	801.2143	1078.22471	0.7431					82.28	
		1019.6803	1366.9935	0.7459					82.59	82.44
III	8.17	801.2143	1079.5813	0.7422					82.18	
		1319.3659	1767.4854	0.7465	713.7597	1020.0238	0.6997		82.66	82.42
IV	8.22	1211.0834	1616.5497	0.7492					82.46	
		1351.2059	1806.6207	0.7479					82.31	82.38
V	8.17	1051.4569	1411.2979	0.7450					82.49	
		1162.8036	1562.6447	0.7441					82.40	82.44
Mean										82.43
Standard Deviation (SD)										0.03
Relative Standard Deviation (%RSD)										0.04
Purity of Standard(P)			99.50%	Weight of Std.(W`)		6.40 mg	Dilution Factor			1.00

Typical Calculation

Thiram A.I. Content (%w/w)	Precision(%RSD)
$=\frac{RF \times W` \times P}{RF`ave \times W} \times D$	$=\frac{Standard\ Deviation}{Mean\ Content} \times 100$
$=\frac{0.7430 \times 6.40 \times 99.50}{0.7039 \times 8.15} \times 1.00$ = 82.48 %	$=\frac{0.03}{82.43} \times 100$ = 0.04%

Calculation of Accuracy (%Recovery)

Replication	Actual Thiram Content (%w/w) [A]	Spiked Thiram Content (%w/w) [B]	Total Content After Spiking (%w/w) [A+B]	Actual Recovered Spiked Content (%w/w) [E=C-A]	Accuracy(% Recovery) [E/B× 100]
I	80.54	1.875	82.42	1.924	102.60
II		1.871	82.41	1.899	101.54
III		1.871	82.41	1.883	100.65
IV		1.859	82.40	1.844	99.16
V		1.871	82.41	1.905	101.83
Mean					101.16
Standard Deviation					1.32
Relative Standard Deviation (%RSD)					1.30

Conclusion:-

From the results of the analytical method validation, it is concluded that the analytical method is specific, sensitive, precise and accurate for the analysis of thiram. The method is similarly adaptable as that of single method of analysis of these pesticides and can detect this pesticide simultaneously without compromise in recovery and sensitivity by RP-HPLC-UV method. The recovery, linearity, specificity, accuracy and precision show that method is rapid, accurate and precise for the determination of thiram active contentand hisdifferent types formulation. The obtained results of this above said method shows good accuracy and recovery. The results of validation criteria are within the specified limits of SANCO/3030/99 rev. 4, Dir. 91/414/EEC (2000) and OPPTS 830.1800 guidelines. Finally, we can say that optimized method is consequently useful for both qualitative and quantitative investigation in routine analyses by agrochemicals business and research organizations within acceptable limits.

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